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High Contents of Trimethylamine Oxide Correlating With Depth in Deep-Sea Teleost Fishes, Skates, and Decapod Crustaceans

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Abstract. In muscles of shallow-living marine animals, the osmolyte trimethylamine *N*-oxide (TMAO) is reportedly found (in millimoles of TMAO per kilogram of tissue wet weight) at 30–90 in shrimp, 5–50 in crabs, 61–181 in skates, and 10–70 in most teleost fish. Recently our laboratory reported higher levels (83–211 mmol/kg), correlating with habitat depth, in deep-sea gadiform teleosts. We now report the same trend in muscles of other animals, collected off the coast of Oregon from bathyal (1800–2000 m) and abyssal plain (2850 m) sites. TMAO contents (mmol/kg ± SD) were as follows: zoarcid teleosts, 103 ± 9 (bathyal) and 197 ± 2 (abyssal); scorpaenid teleosts, 32 ± 0 (shallow) and 141 ± 16 (bathyal); rajid skates, 215 ± 13 (bathyal) and 244 ± 23 (abyssal); caridean shrimp, 76 ± 16 (shallow), 203 ± 35 (bathyal), and 299 ± 28 (abyssal); *Chionoecetes* crabs, 22 ± 2 (shallow) and 164 ± 15 (bathyal). Deep squid, clams, and anemones also had higher contents than shallow species. Osmoconformers showed compensation between TMAO and other osmolytes. Urea contents (typically 300 mmol/kg in shallow elasmobranchs) in skates were 214 ± 5 (bathyal) and 136 ± 9 (abyssal). Glycine contents in shrimp were 188 ± 17 (shallow) and 52 ± 20 (abyssal). High TMAO contents may reflect diet, reduce osmoregulatory costs, increase buoyancy, or counteract destabilization of proteins by pressure.

Introduction

There are two different adaptive strategies that allow marine organisms to regulate cell volume in the face of the high osmotic pressure of seawater (about 1000 mosm). First, most marine organisms are osmoconformers, maintaining cellular water balance with certain organic osmolytes, especially polyols, neutral amino acids, and methylamines. Unlike most inorganic ions, organic osmolytes are mostly "compatible" because they raise cellular osmotic pressure without adversely affecting macromolecules (Yancey *et al.*, 1982). Recently, Carr *et al.*, (1996) showed that the types of osmolytes used by fishes, crustaceans, and molluscs correlate highly with taxa; *e.g.*, bivalve molluscs use predominantly taurine and betaine (*N*-trimethylglycine), whereas decapod crustaceans use mainly glycine and betaine. This suggests that many osmolytes are interchangeable in their compatibility properties, and whether there are selective forces for different specific combinations of osmolytes in different animals is uncertain in many cases. One possible selective pressure involves trimethylamine *N*-oxide (TMAO) and related osmolytes, which are termed "counteracting" (Yancey *et al.*, 1982) or "compensatory" (Gilles, 1997) because they can enhance the binding activity and stability of proteins and offset the adverse effects of salt ions, high temperature, and the osmolyte urea in cartilaginous fish (Yancey, 1994). In the literature, TMAO is reported to be low or absent in many taxa and found at the highest amounts in the muscles of crustaceans (up to 90 mmol/kg wet wt. in decapod shrimp), squids (up to 140 mmol/kg), and cartilaginous fishes (up to 180 mmol/kg in skates) (Dyer, 1952; Hebard *et al.*, 1982).

The second osmotic strategy is hypo-osmotic regulation, exemplified by marine teleost fishes (except for some polar fishes with high glycerol; Raymond, 1994). With internal fluids at 300–400 mosm (Lange and Fugelli, 1965), most teleosts should have no need to accumulate large amounts of organic osmolytes. They generally contain TMAO, but until recently the levels reported were only 10–70 mmol/kg

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Abbreviations: TMAO, trimethylamine *N*-oxide; TMA, trimethylamine

muscle, with gadiform fishes (cods and relatives) having the highest amounts (Hebard *et al.*, 1982). Recently, however, exceptions have been found: some polar fishes have TMAO contents up to 154 mmol/kg muscle (Raymond and DeVries, 1998); and our laboratory has found high levels (83–211 mmol/kg muscle) in deep-sea gadiform teleosts, with abyssal species having more than bathyal species (Gillett *et al.*, 1997).

Why should deep-sea osmoregulators need to retain such large amounts of TMAO? We hypothesized that high levels of TMAO might lower the energetic costs of hypo-osmoregulation in the energy-poor deep sea or stabilize proteins under high hydrostatic pressure, which is known to affect protein activity and structure adversely (Siebenaller, 1987; Balny *et al.*, 1997). Although some enzymes in deep-sea organisms function normally under high pressures, presumably because of structural adaptations in the proteins, others exhibit significant pressure sensitivities (Siebenaller, 1987). The previous study in our laboratory found that, for macrourid lactate dehydrogenase, the Michaelis constant for cofactor was increased by high pressure, but decreased with the addition of TMAO (Gillett *et al.*, 1997). A third hypothesis is based on calculations that TMAO solutions are more buoyant than solutions of other osmolytes (Withers *et al.*, 1994), though not more buoyant than pure water. Thus TMAO might increase buoyancy in an osmoconformer, but not in a hypo-osmoregulator.

To elucidate further the possible roles of TMAO in the deep sea, in this study we analyzed TMAO contents in other families of teleosts and in osmoconforming animals, which presumably would not save osmoregulatory energy by using TMAO in place of other osmolytes.

Materials and Methods

Species analyzed

A complete list of species used in this study is given in Table 1. Deep-sea animals were caught by otter trawl off the Oregon coast in April 1996 and 1997. Two sites were trawled from the R/V *Wecoma:* one was on the abyssal plain at 2850 m (44°46′N, 125°39′W) and the other was a bathyal site on the continental slope at 1800–2000 m (44°28′N, 125°08′W). Samples were taken from the dorsal white muscle of teleost fish, wing muscle of skates, abdominal muscle of shrimp, leg muscle of crab, mantle of squid, and internal septal tissue of anemones.

Similar tissue samples were taken from a variety of shallow-living animals, most of which were collected north of Lopez Island, Washington State, in March 1998. These animals were caught by otter trawl at 40–80 m from the R/V *Nugget.* Fresh specimens of a few species (see Table 1) were purchased at the Pike Street Fishmarket in Seattle, Washington, in June 1998. In addition, G. Somero from Hopkins Marine Station, Pacific Grove, California, kindly provided fresh specimens of *Pachygrapsus crassipes* (a brachyuran crab) and *Squalus acanthias* (the dogfish shark). These two species (not listed in Table 1) were used to provide additional data for comparison (see Results).

Table 1

Species, with depth range (if known), used to compare content of trimethylamine N-oxide (TMAO) in tissue of marine animals from three depth habitats*

Shallow	Bathyal (1800–2000 m)	Abyssal (2850 m)
Teleost fishes—gadiform families Gadidae, Macrouridae (cods, grenadiers)		
Gadus macrocephalus (12–549 m)	*Coryphaenoides cinereus* (225 –2830 m)	*Coryphaenoides armatus* (2000–5200 m)
Teleost fishes—gadiform family Moridae (morid cod)		
	Antimora microlepis (530–2940? m)	*Antimora microlepis* (530–2940? m)
Teleost fishes—scorpaeniform family Scorpaenidae (rockfish etc.)		
Sebastes melanops† (0–366 m)	*Sebastolobus altivelis* (305 to >1500 m)	
Teleost fishes—ophidiiform family Zoarcidae (zoarcid eels)		
	Lycenchelys sp.	*Pachycara* sp.
Elasmobranch fishes—rajiform family Rajidae (skates)		
	Bathyraja spinosissima (1280–1829 m)	*Bathyraja* unnamed‡ (>2500 m)
Decapod crustaceans—suborder Caridea (shrimp)		
Pandalus danae	*Pandalopsis ampla*	*Neocrangon abyssorum*
Decapod crustaceans—decapod suborder Brachyura (crabs)		
Chionoecetes bairdi	*Chionoecetes angulatus*	
Cephalopod molluscs—families Loliginidae, Gonatidae, Onychoteuthidae (squid)		
Loligo opalescens† (inner shelf to surface)	*Berryteuthis magister* (30–1500 m near bottom)	*Gonatus borealis* (epipelagic-abyssal) *Moroteuthis robusta* (subtidal-bathyal?)
Pelecypod molluscs—eulamellibranch family Veneridae, septibranchia family Cuspidaridae		
Saxidomus giganteus†	*Cuspidaria glacialis*	*Cuspidaria glacialis*
Anthozoan cnidaria—order Actiniaria		
Urticina lofotensis	*Actinauge abyssorum*	*Actinauge abyssorum*

* From Eschmeyer *et al.* (1983); Pearcy *et al.* (1982); Allen and Smith (1988); Nesis (1987).

† Specimens obtained from Pike's Fishmarket, Seattle.

‡ Close relative of *Bathyraja trachura* (Eschmeyer *et al.*, 1983).

Analytical procedures

Samples were frozen at −80°C on the ships, transported to Whitman College on dry ice, and stored at −70°C. Muscle was processed with perchloric acid, then neutralized and filtered as previously described (Wolff *et al.*, 1989). The TMAO content was determined according to the procedure of Wekell and Barnett (1991), by reducing TMAO to trimethylamine (TMA) with an iron-EDTA reagent. TMA was extracted in toluene and reacted with 0.02% picric acid; the

colored product was measured spectrophotometrically. Standards were treated similarly with each run. The TMA content of tissues, which was <3 m*M* in all cases, was also measured by omitting the reduction step; the results were subtracted from the TMAO values. Sugars, neutral amino acids, and urea were analyzed in extracts by high performance liquid chromatography using a Waters Sugarpak column as previously described (Wolff *et al.,* 1989).

Statistical analysis of the osmolyte contents was performed with analysis of variance (ANOVA) followed by the Student-Newmann-Keuls post-test.

Results

We analyzed the TMAO content of muscles of deep-living fishes, crustaceans, squids, and clams and compared the results with published data for shallow-living species. Published values have been generally consistent over many decades, but they have been obtained by different techniques (Hebard *et al.,* 1982); therefore, whenever possible, we also analyzed fresh tissue from related shallow-living species for comparison.

In general, the TMAO content of tissue increased significantly with the depth at which the animal was caught—in the order of shallow < bathyal < abyssal (Table II). The results for fishes and crustaceans are shown in Figure 1. We initially confirmed our previous finding of this trend in gadiform teleosts (shallow Gadidae and deep Macrouridae and Moridae). For the gadid and macrourids, the trend was found among different species at each depth. However, the morid *Antimora microlepis* was caught at both depths, and the single animal from the abyssal site had much higher amounts of TMAO than did the same species from the bathyal site (288 mmol/kg wet wt. compared to 211; Table II, Fig. 1).

Similar patterns occurred in two other, not closely related teleost families. Values in the literature for the TMAO contents of shallow Scorpaenidae are relatively low (Hebard *et al.,* 1982). The value of 32 mmol/kg we found in shallow *Sebastes melanops* was well within this range, and was much less than the 141 mmol/kg we measured for the bathyal *Sebastolobus altivelis* (Table II, Fig. 1). No shallow species of Zoarcidae were available to us; however, a published value of 51 mmol/kg for one species (Dyer, 1952) was much less than the value of 103 for the bathyal species, which in turn was less than the 197 for the abyssal species (Table II, Fig. 1).

Among cartilaginous fishes, only deep Rajidae (skates) in the genus *Bathyraja* were caught. In the literature, many species in the closely related genus *Raja* have been found to contain TMAO in muscle over a wide range, from 61–181 mmol/kg (Hebard *et al.,* 1982). The TMAO content was higher in the deep-living *Bathyraja* species, although the values for bathyal and abyssal specimens (215 and 244 mmol/kg, respectively) were not quite significantly different from each other (Table II, Fig. 1).

The depth trend in decapod crustaceans is also shown in Figure 1 for caridean shrimp and brachyuran crabs. For the shallow shrimp *Pandalus danae*, the average TMAO of 76 mmol/kg fell in the published range for many caridean species, while averages were significantly higher in bathyal and abyssal species (203 and 299 mmol/kg, respectively; Table II, Fig. 1). The value of 22 mmol/kg for the shallow subtidal snow crab *Chionoecetes bairdi* fell in the published range for several brachyuran species (Table II, Fig. 1). Similarly, the intertidal crab *Pachygrapsus crassipes* gave a value of 20 ± 5 mmol/kg (n = 3) (not shown in tables or figures). Again, a bathyal species, the congener snow crab

Table II

Comparison of trimethylamine N-oxide (TMAO) content (mmol TMAO/kg tissue wet weight) of tissues of marine animals from three depth habitats

	Habitat			
Data Source	Shallow	Bathyal (1800–2000 m)	Abyssal (2850 m)	*P* value
Teleost fishes—cods and grenadiers (muscle)				
This study	46 ± 2 (2)	133 ± 19 (5)	179 ± 12 (8)	<0.001
Published	66 ± 4 (6)*			
Teleost fishes—morid cods (muscle)				
This study		211 ± 18 (4)	288 (1)	
Teleost fishes—scorpaenids (muscle)				
This study	32.2 ± 0 (2)	141 ± 16 (5)		<0.001
Published	6–64†			
Teleost fishes—zoarcids (muscle)				
This study	—	103 ± 9 (3)	197 ± 2 (2)	<0.001
Published	51 ± 5 (2)§			
Elasmobranch fishes—skates (muscle)				
This study		215 ± 13 (4)	244 ± 23 (4)	0.075
Published	61–181†			
Decapod crustaceans—caridean shrimp (muscle)				
This study	76 ± 19 (5)	203 ± 35 (7)	299 ± 28 (5)	<0.001
Published	30–90†			
Decapod crustaceans—brachyuran crabs (muscle)				
This study	22 ± 2 (2)	164 ± 15 (3)		<0.001
Published	5–50†			
Cephalopod molluscs—squids (mantle)				
This study	48 ± 7 (3)	219 (1)	333, 377	
Published	30–140†			
Pelecypod molluscs—venerid and cuspidarid (siphons)				
This study	3 ± 5 (3)	46 (1)	134 ± 20 (2)	
Published	0–50†			
Anthozoan cnidaria (internal septa)				
This study	0 (3)	10.6 ± 0.6 (3)	31.6 ± 3.3 (3)	<0.001

Note: TMAO values are means (n value in parentheses) ± SD. See Table 1 for species used in this study.

* *Theragra chalcogramma* (Alaskan pollock) analyzed by Wekell and Barnett (1991).

† Numerous species reviewed by Hebard *et al.* (1982).

§ *Macrozoarces americanus* (depth 10–180 m) analyzed by Dyer (1952).

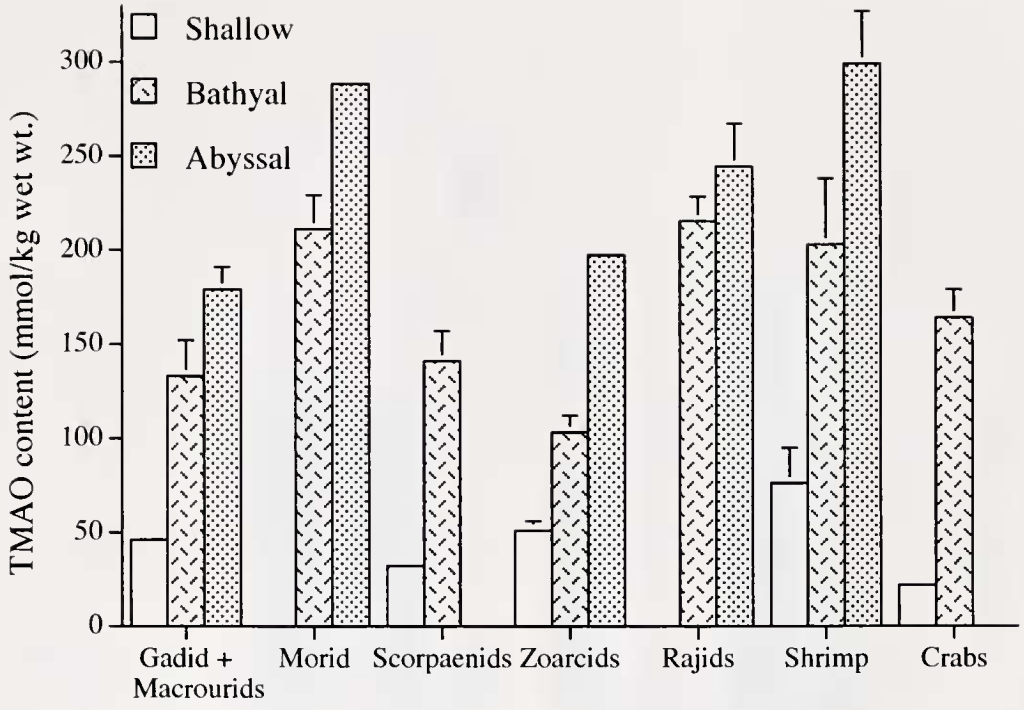

Figure 1. Trimethylamine *N*-oxide (TMAO) content (from Table II) in muscles of marine animals from shallow habitats and bathyal (1800–2000 m) and abyssal (2850 m) trawl sites (see Table I for species). Error bars indicate SD. All differences between depths within each animal type were significant except for the morid (n = 1 for abyssal) and the rajids (see Table II).

C. angulatus, had much higher levels at 164 mmol/kg (Table II, Fig. 1).

For deep-living squid, only one specimen each of three species was caught. The TMAO contents were highest (333, 377 mmol/kg) in the two species caught in the abyssal trawls, moderate (219) in the species from a bathyal trawl, and low (48) in the shallow-living *Loligo* squid (Table II), within the published range of 35–55 mmol/kg for other Loliginidae (Carr *et al.*, 1996). However, the actual depth of capture could not be determined for the deep squid, which have been found from the deep-sea bottom to the epipelagic zone (Nesis, 1987).

Only two specimens of abyssal and one of bathyal clams (Cuspidaridae) were obtained; for this group and the anemones, no closely related shallow species are known. The abyssal (though not the bathyal) clams had much higher TMAO contents (134 mmol/kg) than reported in the literature for shallow-living pelecypods: negligible in oysters, mussels, clams, and cockles, and up to 50 mmol/kg in scallop muscles (Table II; Hebard *et al.*, 1982). Similarly, a shallow anthozoan anemone had no detectable TMAO, whereas the deeper species had significant amounts correlating with depth (Table II); of note is that the tissue used was muscle, epithelia, and very watery, gelatinous mesoglea with presumably low intracellular space, suggesting that the actual cellular TMAO levels were much higher.

For the osmoconforming animals (skates, decapods), the higher amounts of TMAO should be compensated for by lower amounts of other osmolytes. In the literature, the dominant osmolyte in cartilaginous fishes is urea at about 300 mmol/kg average, with TMAO generally the second most concentrated. Most studies show urea in a content ratio of 3:1 or 2:1 (about 2:1 intracellular) to TMAO and other methylamines such as betaine and sarcosine (Yancey, 1985). As another check on our methodology, we measured TMAO in a shallow elasmobranch, the Pacific dogfish *Squalus acanthias*, finding TMAO at 158 and urea at 301 mmol/kg (n = 1). This agreed well with published data for the North Sea *S. acanthias* (144 and 315 mmol/kg, respectively; Vyncke, 1970). In the deep *Bathyraja* species, we found a significant depth trend ($P < 0.001$) of decreasing urea content: 214 ± 5 mmol/kg (bathyal) and 136 ± 8.6 mmol/kg (abyssal), with urea:TMAO ratios of approximately 1:1 and 1:2, respectively (Fig. 2).

In shallow decapods, glycine is the dominant osmolyte (Carr *et al.*, 1996). We confirmed this in shallow caridean shrimp (188 ± 17 mmol/kg glycine), but found considerably less glycine (52 ± 20; $P < 0.001$), along with the much higher levels of TMAO (299), in the abyssal species (Fig.

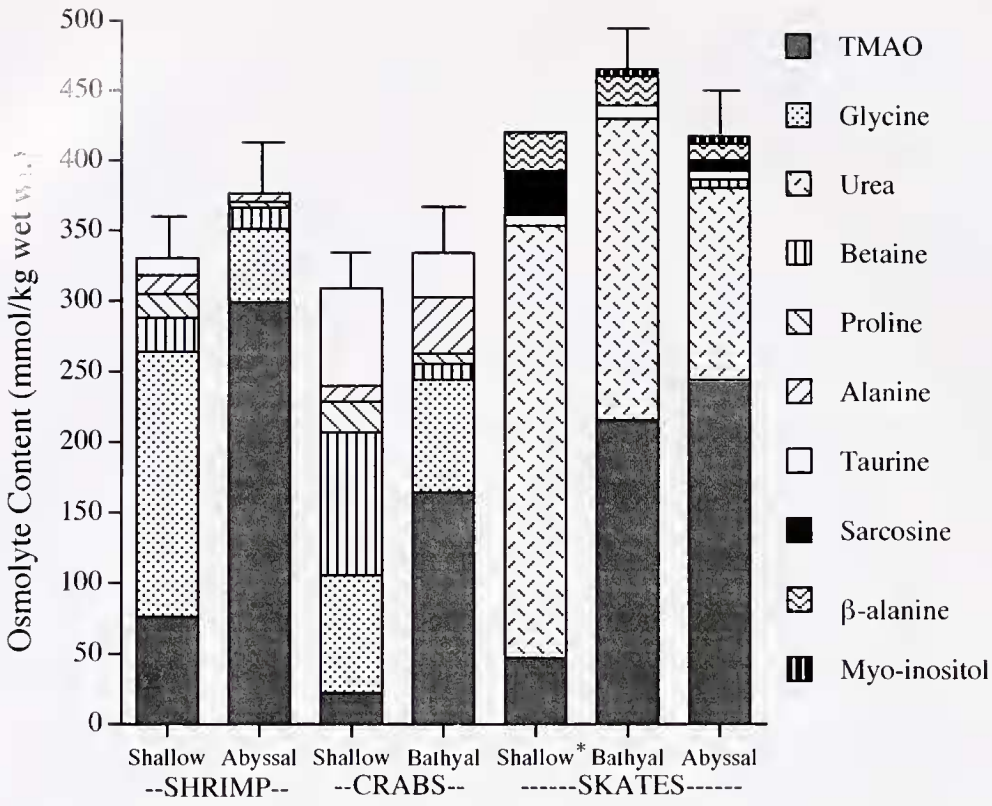

Figure 2. Osmolyte content in muscles of marine animals from shallow habitats and bathyal (1800–2000 m) and abyssal (2850 m) trawl sites (see Table I). Trace amounts of other amino acids are not shown. Error bars indicate SD for summed osmolytes; totals were not significantly different between animals of the same type. **Raja erinacea* data calculated from Forster and Goldstein (1976) and King *et al.* (1980).

2). Other minor osmolytes were also lower in the abyssal specimens; the overall sum of the major osmolytes detected are shown in Figure 2. Similarly, shallow crabs were dominated by glycine, betaine, and taurine, the latter two of which were considerably less ($P < 0.001$) in the bathyal species (Fig. 2).

Discussion

This study greatly extends our previous finding (Gillett *et al.*, 1997) that there is a significant trend of increasing contents of TMAO in muscles with habitat depth. The pattern has now been found among three teleost families of the order Gadiformes (a gadid, a morid, and a macrourid *Coryphaenoides* congener); possibly within the morid species *Antimora microlepis;* within two other teleost families—the Zoarcidae (order Ophidiiformes) and the Scorpaenidae (order Scorpaeniformes); and in the crustacean order Decapoda within the suborders Caridae and Brachyura, including congeners in the latter. In the elasmobranch Rajidae, although the average TMAO content in the abyssal species was not quite significantly greater than in the bathyal one, both were well above that previously reported for rajiform skates (Table II). The data for clams and anemones were also consistent with the trend, though interpretation is limited by the lack of close shallow relatives. Squids caught in deep trawls also had much higher TMAO levels than shallow species (Table II), but limited specimen numbers, distant relationships, uncertain capture depths, and poorly known habitat ranges make significant conclusions impossible.

We have previously shown that TMAO concentrations are quite low in plasma in the macrourid teleosts; thus a whole-tissue content of 170 mmol/kg should be equivalent to an intracellular concentration of perhaps 250 m*M* TMAO. However the morid cod (*A microlepis*) had very high plasma TMAO (159 m*M*); thus its muscle content of 211 mmol/kg (bathyal) would correspond to 250–300 m*M* intracellular also (Gillett *et al.*, 1997). Interestingly, the one *A. microlepis* caught at the abyssal site contained much higher amounts of TMAO than individuals of the same species caught from the bathyal site (Table II, Fig. 1). This species is thought to migrate between continental slopes and abyssal

depths (Allen and Smith, 1988). The possibility that individuals within a species could be regulating TMAO levels at different depths requires further study.

These patterns suggest that a high TMAO content is an adaptive response in animals to living at great depths. We are examining several hypotheses.

Diet

For many species, high concentrations of TMAO could simply reflect diet; if, for example, shrimp or smaller animals low in the food chain have high TMAO and are eaten by the other animals in this study. However, that would leave open the questions of why TMAO is high in lower trophic levels, and why hypo-osmoregulators retain it.

Hypo-osmoregulation costs

Because hypo-osmoregulation is energetically costly (Griffith and Pang, 1979), a greater amount of TMAO may be an adaptation to conserve energy in the low-energy environment of the deep sea. Higher concentrations of solute in tissue might decrease the amount of energy needed to actively transport ions in deep-sea osmoregulators. TMAO could also be used to detoxify ammonia if water is limiting (Dyer, 1952). However, recent calculations by Kirschner (1993) suggest that hypo-osmoregulating is no more metabolically expensive than osmoconforming. Also, if a higher osmotic content saves energy, it is not clear why most shallow-living teleosts have not evolved such a strategy. The issue remains unresolved. In any case, hypo-osmoregulation costs would not apply to osmoconforming skates and invertebrates in our study.

Buoyancy

A third hypothesis is based on calculations that dissolved TMAO is more buoyant (1000 g/ml for 1 *M* solution) than common physiological solutes (Withers *et al.*, 1994). TMAO may save energy in shrimp, for example, by replacing less buoyant osmolytes (Fig. 2), especially glycine (1.029 g/ml for 1 *M* solution). High levels of similarly buoyant TMA (128 m*M*) have been reported in the carapace fluid of a deep-sea oplophorid shrimp (*Notostomus gibbosus*) and calculated to increase buoyancy over other ions (Sanders and Childress, 1988). Because TMA is toxic, one might predict it to be low in cells, but *N. gibbosus* has not been tested for tissue TMA (or TMAO) content. However, we did find TMA concentrations to be less than 3 mmol/kg in muscle extracts (which include intra- and extracellular fluids) for all of our specimens.

Interestingly, some pelagic squid (such as cranchiids) reportedly have high amounts of ammonium ions in their fluids for buoyancy (Nesis, 1987). However, Sanders and Childress (1988) note that the tests used did not discriminate between ammonium and TMA. High fluid TMA would correspond with our finding of high muscle TMAO in oceanic squid (Table II). The squids in this study are vertical migrators and could readily benefit from TMAO buoyancy. But if this is the case, the question remains why epipelagic animals (*e.g.*, shallow shrimp, Fig. 2) do not have higher amounts. Finally, the deep-sea skates, crabs, clams, and anemones with higher TMAO levels are primarily or fully benthic, so they do not require enhanced buoyancy.

Regardless of its function in shrimp, TMAO should not elevate buoyancy in hypo-osmotic fishes: TMAO solutions have the same buoyancy as pure water, which is effectively what TMAO replaces in these fish compared to shallow-water relatives (with their lower osmotic pressures; Gillett *et al.*, 1997). One possibility is that the density of TMAO solutions might decrease relative to pure water at high hydrostatic pressure, but that has not been measured. Finally, grenadiers are demersal, but scorpaenids are primarily benthic and thus probably do not require enhanced buoyancy.

Temperature

Another characteristic of deep habitats is cold temperatures. The depth trend in TMAO content may reflect some property of the osmolyte that is of more selective value in the cold. For example, another methylamine osmolyte, β-dimethylsulfoniopropionate, is a better stabilizer in the cold (Nishiguichi and Somero, 1992), but TMAO has not been tested extensively for this property. Raymond and DeVries (1998) have shown that some Antarctic teleosts have high levels (74–154 mmol/kg muscle) of TMAO as well, and they hypothesize that it either acts as an antifreeze or counteracts salt effects on proteins (see below). However, the temperatures at our deep sampling sites were about 2° (abyssal) to 3°C (bathyal) (Kennish, 1989). Since these are above freezing, and since TMAO levels are not particularly high in many shallow, cold-adapted fish (*e.g.*, the gadid Alaskan pollock [Table II; Wekell and Barnett, 1991]), an antifreeze function can be ruled out for our species. Moreover, TMAO contents were roughly linearly correlated with depth but temperatures are not, so the temperature hypothesis is further weakened.

Macromolecular stabilization

The final hypotheses are based on the well-documented protein-stabilizing capabilities of TMAO; for example, TMAO in elasmobranchs (up to 200 mmol/kg) can counteract the destabilizing effects of urea, their main and non-compatible osmolyte (Yancey, 1985, 1994). Recently, it has also been shown that TMAO can rescue misfolded proteins of medical importance such as the cystic fibrosis transmembrane conductance regulator (Welch and Brown, 1996). We have hypothesized that high TMAO levels may counteract

the adverse affects of hydrostatic pressure on proteins in deep-sea teleosts (Gillett *et al.*, 1997), and Raymond and DeVries (1998) have proposed counteraction of the effects of elevated NaCl in Antarctic teleosts. Testing of these hypotheses has just begun; we did find that at 300 atm, the inhibition of cofactor binding by lactate dehydrogenase from a macrourid teleost was fully offset with 250 m*M* TMAO (Gillett *et al.*, 1997). We are currently studying the effects of TMAO on other proteins at high pressure.

The stabilization hypothesis is further supported by the finding of reduced glycine in deep-sea shrimp and urea in skates, along with higher TMAO (Fig. 2). Glycine does not generally have as great a protein-stabilizing capability as TMAO (Yancey, 1994). Similarly, urea is a protein destabilizer, and its ratio to TMAO is reversed in abyssal skates compared to most shallow species (Fig. 2). Indeed, to our knowledge the urea values for the abyssal skate are the lowest yet reported for a marine cartilaginous fish. Of course, it is possible that substantial urea was lost from deep skates during their 90- to 120-min trips to the surface. As evidence against this urea loss, skates ranged in size from 10–90 cm, but the standard deviations were quite low (6% in abyssal, 2% in bathyal).

The stabilization hypothesis could be valid for all species examined here. Wang and Bolen (1997) have shown that unfavorable interactions between TMAO and peptide backbones stabilize protein structure, supporting earlier hypotheses that such osmolyte effects are universal (Yancey *et al.*, 1982). The effect on proteins under pressure remains speculative, but we know that destabilizing inorganic ions alter water structure in a manner essentially identical to pressure effects (Leberman and Soper, 1995). Perhaps compensatory osmolytes do the opposite (Gillett *et al.*, 1997).

It is possible that all of these hypotheses are correct but only one or two apply to each group of animals. In conclusion, this study suggests that osmolyte properties may play a far more important role in adaptation to specific marine habitats than previously known.

Acknowledgments

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Literature Cited

Allen, M. J., and G. B. Smith. 1988. Atlas and zoogeography of common fishes in the Bering Sea and northeastern Pacific. *NOAA Technical Report NMFS*, 66. U.S. Dept. Commerce, Springfield, VA.

Balny, C., V. V. Mozhaev, and R. Lange. 1997. Hydrostatic pressure and proteins: basic concepts and new data. *Comp. Biochem. Physiol.* **116A:** 299–304.

Carr, W. E. S., J. C. Netherton III, R. A. Gleeson, and C. D. Derby. 1996. Stimulants of feeding behavior in fish: analyses of tissues of diverse marine organisms. *Biol Bull.* **190:** 149–160.

Dyer, W. J. 1952. Amines in fish muscle. VI. Trimethylamine oxide content of fish and marine invertebrates. *J. Fish. Res. Board. Can.* **8:** 314–324.

Eschmeyer, W. N., E. S. Herald, and H. Hammann. 1983. *A Field Guide to Pacific Coast Fishes of North America.* Houghton Mifflin, Boston.

Forster, R. P., and L. Goldstein. 1976. Intracellular osmoregulatory role of amino acids and urea in marine elasmobranchs. *Am. J. Physiol.* **230:** 925–931.

Gilles, R. 1997. "Compensatory" organic osmolytes in high osmolarity and dehydration stresses: history and perspectives. *Comp. Biochem. Physiol.* **117A:** 279–290.

Gillett, M. B., J. R. Suko, F. O. Santoso, and P. H. Yancey. 1997. Elevated levels of trimethylamine oxide in muscles of deep-sea gadiform teleosts: a high-pressure adaptation? *J. Exp. Zool.* **279:** 386–391.

Griffith, R. W., and P. K. T. Pang. 1979. Mechanisms of osmoregulation of the coelacanth: evolutionary implications. *Occas. Pap. Calif. Acad. Sci.* **134:** 79–92.

Hebard, C. E., G. J. Flick, and R. E. Martin. 1982. Occurrence and significance of trimethylamine oxide and its derivatives in fish and shellfish. Pp. 149–175 in *Chemistry and Biochemistry of Marine Food Products*, R. E. Martin, ed. AVI Publishing, Westport, CT.

Kennish, M. J. 1989. *Practical Handbook of Marine Science.* CRC Press, Boca Raton, FL.

King, P. A., C-J. Cha, and L. Goldstein. 1980. Amino acid metabolism and cell volume regulation in the little skate, *Raja erinacea*. *J. Exp. Zool.* **212:** 79–86.

Kirschner, L. B. 1993. The energetics of osmotic regulation in ureotelic and hypoosmotic fishes. *J. Exp. Zool.* **267:** 19–26.

Lange, R., and K. Fugelli. 1965. The osmotic adjustment in the euryhaline teleosts, the flounder, *Pleuronectes flesus* L., and the three-spined stickleback, *Gastrosteus aculaetus* L. *Comp. Biochem. Physiol.* **15:** 283–292.

Leberman, R., and A. K. Soper. 1995. Effect of high salt concentrations on water structure. *Nature* **378:** 364–367.

Nesis, K. N. 1987. *Cephalopods of the World.* T. F. H. Publications, Neptune City, NJ.

Nishiguichi, M. K., and G. N. Somero. 1992. Temperature- and concentration-dependence of compatibility of the organic osmolyte β-dimethylsulfoniopropionate. *Cryobiology* **29:** 118–123.

Pearcy, W. G., D. L. Stein, and R. S. Carney. 1982. The deep-sea benthic fish fauna of the northeastern Pacific Ocean on Cascadia and Tufts abyssal plains and adjoining continental slopes. *Biol. Oceanogr.* **1:** 375–428.

Raymond, J. A. 1994. Seasonal variations of trimethylamine oxide and urea in the blood of a cold-adapted marine teleost, the rainbow smelt. *Fish Physiol. Biochem.* **13:** 13–22.

Raymond, J. A., and A. L. DeVries. 1998. Elevated concentrations and synthetic pathways of trimethylamine oxide and urea in some teleost fishes of McMurdo Sound, Antartica. *Fish Physiol. Biochem.* **18:** 387–398.

Sanders, N. K., and J. J. Childress. 1988. Ion replacement as a buoyancy mechanism in a pelagic deep-sea crustacean. *J. Exp. Biol.* **138:** 333–343.

Siebenaller, J. F. 1987. Pressure adaptation in deep-sea animals. Pp.

33–48 in *Current Perspectives in High Pressure Biology*. H. W. Jannasch, R. E. Marquis, and A. M. Zimmerman, eds. Academic Press, London.

Vyncke, W. 1970. Influence of biological and environmental factors on nitrogenous extractives of the spurdog *Squalus acanthias*. *Mar. Biol.* **6:** 248–255.

Wang, A., and D. W. Bolen. 1997. A naturally occurring protective system in urea-rich cells: mechanism of osmolyte protection of proteins against urea denaturation. *Biochemistry* **36:** 9101–9108.

Wekell, J. C., and H. Barnett. 1991. New method for analysis of trimethylamine oxide using ferrous sulfate and EDTA. *J. Food Sci.* **56:** 132–138.

Welch, W. J., and C. R. Brown. 1996. Influence of molecular and chemical chaperones on protein folding. *Cell Stress Chaperones* **1:** 109–115.

Withers, P. C., G. Morrison, G. T. Hefter, and T.-S. Pang. 1994. Role of urea and methylamines in buoyancy of elasmobranchs. *J. Exp. Biol.* **188:** 175–189.

Wolff, S., P. H. Yancey, T. S. Stanton, and R. Balaban. 1989. A simple HPLC method for quantitating the major organic solutes of the renal medulla. *Am. J. Physiol.* **256:** F954–956.

Yancey, P. H. 1985. Organic osmotic effectors in cartilaginous fishes. Pp. 424–436 in *Transport Processes, Iono- and Osmoregulation*, R. Gilles and M. Gilles-Ballien, eds. Springer-Verlag, New York.

Yancey, P. H. 1994. Compatible and counteracting solutes. Pp. 81–109 in *Cellular and Molecular Physiology of Cell Volume Regulation*, K. Strange, ed. CRC Press, London.

Yancey, P. H., M. E. Clark, S. C. Hand, R. D. Bowlus, and G. N. Somero. 1982. Living with water stress: Evolution of osmolyte systems. *Science* **217:** 1214–1222.